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(54) Title: HOMOLOGS OF A PNEUMOCOCCAL PROTEIN AND FRAGMENTS FOR VACCINES



(57) Abstract: The invention is directed to isolated polypeptides bearing sequence homology to the Sp36 protein found in pneumococcal organisms, such as *Streptococcus pneumoniae*. Polynucleotides encoding such polypeptides are also disclosed. The invention also relates to antibodies specific for the disclosed polypeptides and to uses of such antibodies in the treatment of diseases caused by staphylococci as well as group A and B streptococci. In addition, the invention relates to the use of the disclosed polypeptides in compositions and as vaccines and for prophylactic uses such as in vaccination of animals, especially humans, against a wide variety of streptococcal, staphylococcal and other diseases.

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**HOMOLOGS OF A PNEUMOCOCCAL PROTEIN AND FRAGMENTS FOR VACCINES**

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This application claims the priority of U.S. Provisional Application 60/150,750, filed August 25, 1999, the disclosure of which is hereby incorporated by reference in its entirety.

10

**FIELD OF THE INVENTION**

This invention relates generally to the field of bacterial antigens and their use, for example, as immunogenic agents in humans and animals to stimulate an immune response. More specifically, it relates to the vaccination of mammalian species, especially humans, with one or more polypeptides derived from gram positive bacteria and which show sequence homology with an immunogenic polypeptide obtained from *Streptococcus pneumoniae*.

20

**BACKGROUND OF THE INVENTION**

Polypeptides derived from gram positive bacteria are useful for stimulating production of antibodies that protect the vaccine recipient against infection by a wide range of serotypes of pathogenic gram positive bacteria, including *S. pneumoniae*. Further, the invention relates to antibodies against such polypeptides useful in diagnosis and passive immune therapy with respect to diagnosing and treating such pneumococcal infections.

The genus *Streptococcus* contains a variety of species responsible for causing disease in mammals, including humans, while also encompassing species that constitute normal flora in humans and other mammals. Among the bacterial species implicated in the etiology of 5 diseases in humans are *S. pyogenes* (part of the group A streptococcal bacteria, herein designated "GAS" for "group A streptococci"), *S. pneumoniae* (referred to as "pneumococcus") and *S. agalactiae* (the group B streptococci or "GBS"). The group A streptococci cause serious diseases such as necrotizing fasciitis, scarlet fever and sepsis, as well as less virulent 10 diseases such as impetigo and pharyngitis. The pneumococci are the most common cause of community-acquired pneumonia and are also responsible for more than half of all cases of otitis media in children. The pneumococci are also the second most common pathogen associated with bacterial meningitis. The group B streptococci are the most prevalent pathogen 15 associated with illness and death among newborns in the United States.

Currently, there are no vaccines available for the prevention of diseases caused by the group A and group B streptococci and presently available pneumococcal vaccines are not effective in children under 2 years 20 of age or in the elderly due to the poor immunogenicity of the capsular carbohydrates that compose the current vaccine. It would therefore be highly advantageous to produce a vaccine that would prevent infection by these classes of pathogen, especially in the age groups mentioned.

25 In addition to the pathogens just described, some bacteria of the genus *Staphylococcus* are also of clinical importance. In fact, two of these are among the leading causes of nosocomial infections (infections acquired while in the hospital). Both *Staphylococcus aureus* and *Staphylococcus epidermidis* readily colonize the skin of healthy individuals and can cause 30 acute disease in patients following immunosuppression or traumatic injury. Infections caused by these species include bacteremia, endocarditis,

osteomyelitis, wound infections and infections associated with indwelling catheters.

*Streptococcus pneumoniae* is a gram positive bacterium that is a major causative agent in invasive infections in animals and humans, such as the aforementioned sepsis, meningitis, and otitis media, as well as lobar pneumonia (Tuomanen, et al. *New England J. of Medicine* 322:1280-1284 (1995)). As part of the infection process, pneumococci readily bind to non-inflamed human epithelial cells of the upper and lower respiratory tract by binding to eukaryotic carbohydrates in a lectin-like manner (Cundell et al., *Micro. Path.* 17:361-374 (1994)). Conversion to invasive pneumococcal infections for bound bacteria may involve the local generation of inflammatory factors which may activate the epithelial cells to change the number and type of receptors on their surface (Cundell, et al., *Nature*, 377:435-438 (1995)). Apparently, one such receptor, platelet activating factor (PAF) is engaged by the pneumococcal bacteria and within a very short period of time (minutes) from the appearance of PAF, pneumococci exhibit strongly enhanced adherence and invasion of tissue. Certain soluble receptor analogs have been shown to prevent the progression of pneumococcal infections (Idanpaan-Heikkila et al., *J. Inf. Dis.*, 176:704-712 (1997)). A number of other proteins have been suggested as being involved in the pathogenicity of *S. pneumoniae*.

*Streptococcus pneumoniae* itself has been shown to contain a gene which encodes a protein designated herein as Sp36. This protein has a predicted molecular mass of 91,538 Da and contains 5 histidine triad motifs (proposed to be involved in metal binding). The gene encoding this protein appears to be present the 23 serotypes comprising the current commercially available pneumococcal-capsular vaccine. Immunization of mice with this protein, in the presence of Freund's adjuvant, stimulates an immune

response which protects these mice from an intraperitoneal challenge with a dose of virulent pneumococci that would normally kill the mice.

For the reasons already stated above, there not only remains a need  
5 for identifying polypeptides having epitopes in common from various strains  
of *S. pneumoniae* but also from a broader spectrum of gram positive  
bacteria in order to utilize such polypeptides as vaccines to provide  
protection against a wide variety of infectious organisms.

10

#### BRIEF SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided vaccines  
that include polypeptides obtained from gram positive bacteria other than *S.*  
*pneumoniae*, as well as variants of said polypeptides and active fragments  
15 of such polypeptides.

The present invention is also directed to novel genes, and the  
polypeptides encoded thereby, derived from gram positive bacteria other  
than *S. pneumoniae*, and which bear sequence homology to the Sp36 gene  
20 already described. Such gram positive bacteria include the group A and B  
streptococci, as described herein, as well as species of the genus  
*Staphylococcus*, especially *S. aureus*.

In a particular embodiment, the present invention is directed to  
25 specific gene sequences, and proteins encoded thereby, derived from the  
group A and group B streptococci, and to the use of such expressed  
polypeptides and proteins as the basis for pharmaceutical compositions  
useful as vaccines and as a means for enabling isolation of antibodies with  
therapeutic and/or prophylactic activity (such as would be useful in  
30 preparing products like CytoGam).

In a further embodiment, the present invention also relates to the preparation and use of fragments of the novel polypeptides disclosed herein, such fragments being immunogenic in nature and being useful in the preparation of vaccines against diseases caused by the pathogens from which such polypeptides, and fragments thereof, are derived.

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#### BRIEF DESCRIPTION OF DRAWINGS

Figures 1 shows the results of a Southern blot of genomic DNA from *S. aureus*, *S. pyogenes*, and pneumococcus. The DNA was digested with restriction nucleases *Bam*HI or *Pvu*II, and after electrophoresis and transfer to a nylon membrane, was probed with a labeled DNA fragment encompassing the pneumococcal gene encoding Sp36. The hybridization and washes were carried out under low stringency conditions. The results show hybridization by the labeled probe to a *S. aureus* fragment in both the *Bam*HI and *Pvu*II lanes and to two fragments in the *Pvu*II digests of two strains of *S. pyogenes*.

Figures 2 shows an alignment between the Sp36 amino acid sequence from *S. pneumoniae* strain N4 and the homologous sequences from *S. pyogenes* and *S. agalactiae*. Amino acids identical to those of the polypeptide from *S. pneumoniae* are boxed.

Figure 3 shows the results of a Southern blot of genomic DNA from *S. pyogenes*, *S. agalactiae*, and *S. pneumoniae* probed with DNA encoding the full length Sp36 homolog from *S. pyogenes*. The

hybridization was carried out under low stringency conditions. These results demonstrate that the *S. pyogenes* Sp36 homolog, used as a probe, is capable of detecting a homologous gene in *S. agalactiae* and pneumococcus.

5

Figure 4 shows the results of a western blot using rabbit polyclonal antiserum generated against recombinant Sp36 protein cloned from *S. pneumoniae* strain Norway 4. The results demonstrate that this antiserum not only reacts with the protein against which it was raised (here, Sp36),  
10 as well as to a protein of similar size in a lysate of a serotype 6B strain of pneumococcus, but also reacts with a recombinant protein encoded by the Sp36 homolog gene of group B streptococci.

Figure 5 shows the amino acid sequence for the GAS36 homologs  
15 with the histidine triad regions underlined (Fig. 5(a) and (b)) and the sequence for a GBS36 homolog (Fig. 5(c)) with its histidine triad regions underlined.

20

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel polynucleotides and polypeptides derived from species of gram positive bacteria, especially  
25 group A and B streptococci, and including the genus *Staphylococcus*, most especially *S. pyogenes* (GAS), *S. agalactiae* (GBS), and *S. aureus*, respectively.

Further, the present invention is directed to polynucleotides derived  
30 from gram positive bacteria and which are at least partially homologous to

the polynucleotides making up the gene coding for the previously disclosed Sp36 gene of *S. pneumoniae* (U.S. Application Serial No. 60/113,048).

The present invention is also directed to polynucleotides, and  
5 immunologically active fragments, segments, or portions, thereof, which  
polypeptides are encoded by the polynucleotides disclosed herein.

The present invention also relates to such polynucleotides and  
polypeptides in enriched, preferably isolated, or even purified, form.

10

In accordance with the present invention, the term "DNA segment" refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of  
15 contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the segment and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such segments are provided in the form of an open reading frame uninterrupted by internal nontranslated  
20 sequences, or introns, which are typically present in eukaryotic genes. Sequences of non-translated DNA may be present downstream from the open reading frame, where they do not interfere with manipulation or expression of the coding regions.

25

The nucleic acids and polypeptide expression products disclosed according to the present invention, as well as expression vectors containing such nucleic acids and/or such polypeptides, may be in "enriched form." As used herein, the term "enriched" means that the concentration of the material is at least about 2, 5, 10, 100, or 1000  
30 times its natural concentration (for example), advantageously 0.01%, by weight, preferably at least about 0.1% by weight. Enriched preparations

of about 0.5%, 1%, 5%, 10%, and 20% by weight are also contemplated. The sequences, constructs, vectors, clones, and other materials comprising the present invention can advantageously be in enriched or isolated form.

5

"Isolated" in the context of the present invention with respect to polypeptides (or polynucleotides) means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide 10 present in a living organism is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its 15 natural environment. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and most preferably are purified to homogeneity.

The polynucleotides, and recombinant or immunogenic polypeptides, 20 disclosed in accordance with the present invention may also be in "purified" form. The term "purified" does not require absolute purity; rather, it is intended as a relative definition, and can include preparations that are highly purified or preparations that are only partially purified, as those terms are understood by those of skill in the relevant art. For 25 example, individual clones isolated from a cDNA library have been conventionally purified to electrophoretic homogeneity. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Furthermore, claimed polypeptides 30 having a purity of preferably 0.001%, or at least 0.01% or 0.1%; and even 1% by weight or greater is expressly contemplated.

The term "coding region" refers to that portion of a gene which either naturally or normally codes for the expression product of that gene in its natural genomic environment, i.e., the region coding *in vivo* for the native expression product of the gene. The coding region can be from a normal, mutated or altered gene, or can even be from a DNA sequence, or gene, wholly synthesized in the laboratory using methods well known to those of skill in the art of DNA synthesis.

In accordance with the present invention, the term "nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. Generally, DNA segments encoding the proteins provided by this invention are assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

The term "expression product" means that polypeptide or protein that is the natural translation product of the gene and any nucleic acid sequence coding equivalents resulting from genetic code degeneracy and thus coding for the same amino acid(s).

The term "fragment," when referring to a coding sequence, means a portion of DNA comprising less than the complete coding region whose expression product retains essentially the same biological function or activity as the expression product of the complete coding region.

The term "primer" means a short nucleic acid sequence that is paired with one strand of DNA and provides a free 3'OH end at which a DNA polymerase starts synthesis of a deoxyribonucleotide chain.

The term "promoter" means a region of DNA involved in binding of RNA polymerase to initiate transcription.

The term "open reading frame (ORF)" means a series of triplets 5 coding for amino acids without any termination codons and is a sequence (potentially) translatable into protein.

As used herein, reference to a DNA sequence includes both single stranded and double stranded DNA. Thus, the specific sequence, unless 10 the context indicates otherwise, refers to the single strand DNA of such sequence, the duplex of such sequence with its complement (double stranded DNA) and the complement of such sequence.

In accordance with the present invention, the term "percent identity" 15 or "percent identical," when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the sequence to be compared (the "Compared Sequence") with the described or claimed sequence (the "Reference Sequence"). The Percent Identity is then determined according to the following formula:

20

$$\text{Percent Identity} = 100 [1-(C/R)]$$

wherein C is the number of differences between the Reference Sequence 25 and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence wherein (i) each base or amino acid in the Reference Sequence that does not have a corresponding aligned base or amino acid in the Compared Sequence and (ii) each gap in the Reference Sequence and (iii) each aligned base or amino acid in the 30 Reference Sequence that is different from an aligned base or amino acid in the Compared Sequence, constitutes a difference; and R is the number of

bases or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a base or amino acid.

5        If an alignment exists between the Compared Sequence and the Reference Sequence for which the percent identity as calculated above is about equal to or greater than a specified minimum Percent Identity then the Compared Sequence has the specified minimum percent identity to the Reference Sequence even though alignments may exist in which the  
10 hereinabove calculated Percent Identity is less than the specified Percent Identity.

Thus, the present invention is directed to novel, isolated polypeptides comprising an amino acid sequence at least 75% identical to a sequence in  
15 SEQ ID NO: 2, 4 or 6, preferably polypeptides at least 90% identical thereto, more preferably 95% identical to the sequence of SEQ ID NO: 2 or 4, and most preferably having the sequence of either SEQ ID NO: 2 or 4.

The isolated polypeptides of the present invention may be found in a  
20 wide variety of microorganisms, but will commonly be found in an organism selected from the group consisting of group A streptococci, group B streptococci, and *Staphylococcus aureus*, and wherein the group A streptococcal organism is *Streptococcus pyogenes* and the group B streptococcal organism is *Streptococcus agalactiae*. Also, polypeptides of  
25 the invention include, but are in no way limited to, isolated polypeptides having a sequence at least 25% identical to the amino acid sequence of the Sp36 protein of *Streptococcus pneumoniae*.

The present invention further relates to immunogenically active  
30 fragments of the isolated polypeptides disclosed herein.

The terms "fragment," "derivative" and "analog" when referring to the polypeptides disclosed herein means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein, or preprotein, which can be 5 activated by cleavage of the proprotein portion to produce an active mature polypeptide. Such fragments, derivatives and analogs must have sufficient similarity to the polypeptide of SEQ ID NO:2, 4 or 6 so that immunogenic activity of the native polypeptide is retained.

10 The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

15 The fragment, derivative or analog of the polypeptide of SEQ ID NO:2, 4, or 6 may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with 20 another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, 25 derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

30 As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as 5 intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

As used herein with reference to polypeptides, the terms "portion," 10 "segment," and "fragment," refer also to a continuous sequence of residues, such as amino acid residues, which sequence forms a subset of a larger sequence. For example, if a polypeptide were subjected to treatment with any of the common endopeptidases, such as trypsin, chymotrypsin, or papain, the oligopeptides resulting from such treatment would represent 15 portions, segments or fragments of the starting polypeptide.

The present invention is also directed to isolated polynucleotides whose sequences contain coding regions encoding the polypeptides of the present invention, preferably the polypeptides of SEQ ID NO: 2, 4, and 6 20 and most preferably will be the isolated polynucleotides comprising the sequences of SEQ ID NOS: 1, 3, and 5.

The present invention is also directed to fragments or portions of such sequences which contain at least 15 bases, preferably at least 30 25 bases, more preferably at least 50 bases and most preferably at least 80 bases, and to those sequences which are at least 60%, preferably at least 80%, and most preferably at least 95%, especially 98%, identical thereto, and to DNA (or RNA) sequences encoding the same polypeptide as the sequences of SEQ ID NOS: 2, 4, and 6 including fragments and 30 portions thereof and, when derived from natural sources, includes alleles thereof.

Yet another aspect of the present invention is directed to an isolated DNA (or RNA) sequence or molecule comprising at least the coding region of a bacterial gene (or a DNA sequence encoding the same polypeptide as such coding region), in particular an expressed bacterial gene, which bacterial gene comprises a DNA sequence homologous with, or contributing to, the sequence depicted in SEQ ID NOS: 1, 3, and 5 or one at least 60%, preferably at least 80%, and most preferably at least 95%, especially 98%, identical thereto, including 100% identity, as well as fragments or portions of the coding region which encode a polypeptide having a similar function to the polypeptide encoded by said coding region. Thus, the isolated DNA (or RNA) sequence may include only the coding region of the expressed gene (or fragment or portion thereof as hereinabove indicated) or may further include all or a portion of the non-coding DNA (or RNA) of the expressed bacterial gene.

In general, sequences homologous with and contributing to the sequences of SEQ ID NOS: 1, 3, and 5 (or one at least 60%, preferably at least 80%, and most preferably at least 95% identical or homologous thereto) are from the coding region of a bacterial gene.

The polynucleotides according to the present invention may also occur in the form of mixtures of polynucleotides hybridizable to some extent with the gene sequences containing any of the nucleotide sequences of SEQ ID NOS: 1, 3, and 5, including any and all fragments thereof, and which polynucleotide mixtures may be composed of any number of such polynucleotides, or fragments thereof, including mixtures having at least 10, perhaps at least 30 such sequences, or fragments thereof.

30           Fragments of the full length polynucleotide of the present invention may be used as hybridization probes for a DNA library to isolate the full

length DNA and to isolate other DNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 15 bases, may have at least 30 bases and even 50 or more bases. The probe may also be used to identify a DNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of DNA or mRNA to determine which members of the library the probe hybridizes to.

The present invention is also directed to vectors comprising the polynucleotides disclosed herein, as well as to genetically engineered cells comprising such vectors and/or polynucleotides. Thus, the present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

20

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

25  
30

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, 5 nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

10

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those 15 skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be 20 mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda  $P_L$  promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for 25 amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin 30 resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the 5 protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 10 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

15 More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct 20 further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example; Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, phiX174, 25 pBluescript SK, pBSKS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); pTRC99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P<sub>R</sub>, P<sub>L</sub> and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, J., *Basic Methods in Molecular Biology*, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, 5 usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

10

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae Trp1 gene, and a promoter derived from a highly-expressed gene to direct transcription 15 of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK),  $\alpha$ -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a 20 leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

25

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more 30 phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within

the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

5

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017).

10 Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

15 Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

20 Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

25 Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

30 Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by

Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The polypeptides of the present invention, when utilized for clinically related purposes, may also be suspended in a pharmacologically acceptable diluent or excipient to facilitate such uses, which will include

use as a vaccine for the purpose of preventing a wide variety of streptococcal and staphylococcal infections.

In accordance with another aspect of the present invention, there is  
5 provided a vaccine that includes at least one polypeptide that is at least  
75% identical, preferably at least 90% identical and most preferably 95%  
identical, to a polypeptide sequence comprising the sequence of SEQ ID  
NO: 2, 4, or 6. Such variations in homology for putative vaccines are well  
known in the art (See, for example, Hanson et al., "Active and Passive  
10 Immunity Against *Borrelia burgdorferi* Decorin Binding Protein A (DbpA),"  
Infection and Immunity, (May) 1998, p. 2143 – 2153; Roberts et al.,  
"Heterogeneity Among Genes Including Decorin Binding Proteins A and B of  
*Borrelia burgdorferi sensu lato*," Infection and Immunity, (Nov) 1998, p.  
5275-5285). Such observations would similarly apply to portions, segments  
15 or fragments of the polypeptides disclosed herein.

Such segments find a multitude of uses. For example, such segments  
of the polypeptides according to the present invention find use as  
intermediates in the synthesis of higher molecular weight structures also  
20 within the present invention.

The term "active fragment" means a fragment that generates an  
immune response (i.e., has immunogenic activity) when administered, alone  
or optionally with a suitable adjuvant, to an animal, such as a mammal, for  
25 example, a rabbit or a mouse, and also including a human.

In accordance with a further aspect of the invention, a vaccine of the  
type hereinabove described is administered for the purpose of preventing or  
treating infection caused by streptococci and staphylococci as well as many  
30 related organisms.

A vaccine in accordance with the present invention may include one or more of the hereinabove described polypeptides or active fragments thereof. When employing more than one polypeptide or active fragment, such as two or more polypeptides and/or active fragments may be used as 5 a physical mixture or as a fusion of two or more polypeptides or active fragments. The fusion fragment or fusion polypeptide may be produced, for example, by recombinant techniques or by the use of appropriate linkers for fusing previously prepared polypeptides or active fragments.

10 In many cases, the variation in the polypeptide or active fragment is a conservative amino acid substitution, although other substitutions are within the scope of the invention.

15 In accordance with the present invention, a polypeptide variant includes variants in which one or more amino acids are substituted and/or deleted and/or inserted.

20 In another aspect, the invention relates to passive immunity vaccines formulated from antibodies against a polypeptide or active fragment of a polypeptide of the present invention. Such passive immunity vaccines can be utilized to prevent and/or treat streptococcal and staphylococcal infections in patients. In this manner, according to a further aspect of the invention, a vaccine can be produced from a synthetic or recombinant polypeptide of the present invention or an antibody against such 25 polypeptide.

Still another aspect the present invention relates to a method of using one or more antibodies (monoclonal, polyclonal or sera) to the polypeptides of the invention as described above for the prophylaxis and/or treatment of 30 diseases that are caused by streptococcal and staphylococcal bacteria. In particular, the invention relates to a method for the prophylaxis and/or

treatment of infectious diseases that are caused by streptococci and staphylococci. In a still further preferred aspect, the invention relates to a method for the prophylaxis and/or treatment of such diseases as necrotizing fasciitis, scarlet fever, sepsis and many diseases of newborns, in humans  
5 by utilizing a vaccine of the present invention.

Generally, vaccines are prepared as injectables, in the form of aqueous solutions or suspensions. Vaccines in an oil base are also well known such as for inhaling. Solid forms which are dissolved or suspended  
10 prior to use may also be formulated. Pharmaceutical carriers, diluents and excipients are generally added that are compatible with the active ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to, water, saline solutions, dextrose, or glycerol. Combinations of carriers may also be used.

15

Vaccine compositions may further incorporate additional substances to stabilize pH, or to function as adjuvants, wetting agents, or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

20

Vaccines are generally formulated for parenteral administration and are injected either subcutaneously or intramuscularly. Such vaccines can also be formulated as suppositories or for oral administration, using methods known in the art, or for administration through nasal or respiratory routes.

25

The amount of vaccine sufficient to confer immunity to pathogenic bacteria is determined by methods well known to those skilled in the art. This quantity will be determined based upon the characteristics of the vaccine recipient and the level of immunity required. Typically, the amount  
30 of vaccine to be administered will be determined based upon the judgment of a skilled physician. Where vaccines are administered by subcutaneous or

intramuscular injection, a range of 0.5 to 500 µg purified protein may be given.

The present invention is also directed to a vaccine in which a 5 polypeptide or active fragment of the present invention is delivered or administered in the form of a polynucleotide encoding the polypeptide or active fragment, whereby the polypeptide or active fragment is produced *in vivo*. The polynucleotide may be included in a suitable expression vector and combined with a pharmaceutically acceptable carrier.

10

Thus, the present invention expressly contemplates a vaccine composition comprising any of the polypeptides disclosed herein, said polypeptide being present in an amount effective to produce an immune response, and wherein said polypeptide is suspended in a pharmacologically 15 acceptable carrier, diluent or excipient.

The vaccine compositions of the present invention may also comprise live vaccines, containing such organisms as *Streptococcus gordoniae* and *Salmonella typhi*, wherein said organisms contain recombinant polypeptides 20 as disclosed herein.

In addition, the polypeptides of the present invention can be used as immunogens to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in 25 other processes such as affinity chromatography.

Thus, the present invention is also directed to methods for the prevention of a wide variety of diseases caused by streptococcal and staphylococcal organisms, said methods involving the administering of 30 vaccines disclosed herein to animals at risk of such diseases, especially where said animals are humans.

In addition, the invention disclosed herein is also directed to a means of treating animals, especially humans, afflicted with a disease caused by the organisms from which the isolated polypeptides of the 5 invention are derived, such methods including, but not being limited to, administering to an animal, especially a human, afflicted with such a disease of a therapeutically effective amount of an antibody, or mixture of antibodies, against the polypeptides disclosed herein.

10       Antibodies specific for the polypeptides disclosed herein may be either polyclonal or monoclonal and may even be in the form of antisera. When such antibodies are monoclonal in nature, they may be produced by conventional methods of preparing monoclonal antibodies, such as from conventional hybridoma cells, and may also be produced by genetically 15 engineered cells transformed with vectors containing genes specifically coding for the different heavy and light chains of antibody molecules having an arrangement of variable regions specifically complementary to one or more of the polypeptides of the invention. Such recombinantly produced antibodies may be in the form of either dimers or tetramers, 20 depending on the type of cellular expression system utilized therefor.

The invention will now be further described in more detail in the following non-limiting examples and it will be appreciated that additional and different embodiments of the teachings of the present invention will 25 doubtless suggest themselves to those of skill in the art and such other embodiments are considered to have been inferred from the disclosure herein.

### Example 1

#### Southern Blot Analysis of Chromosomal DNA Using Probes Specific for the Sp36 Gene of *Streptococcus pneumoniae*

5

Genomic DNA was isolated from *Staphylococcus aureus*, *Streptococcus pyogenes* (group A), and *Streptococcus agalactiae* (group B) after overnight growth of the bacteria. The DNA was digested to completion by overnight incubation with restriction enzymes (*Bam*HI and 10 *Pvu*II), and then DNA fragments were resolved by size by agarose gel electrophoresis before transfer to a nylon membrane. The membrane was then probed with DNA encoding the entire Sp36 open reading frame that had been fluorescein-labeled with random primers using a kit from Amersham Pharmacia Biotech Inc. The hybridization and washes were 15 carried out under low stringency conditions (i.e., 45°C, 5xSSC hybridization; 45°C, 1xSSC for 1<sup>st</sup> wash; 45°C, 0.5xSSC for 2<sup>nd</sup> wash). Here, SSC is composed of 150 mM NaCl and 15 nM sodium citrate, pH 7.0 and all washes are 50 mL each.

20

After hybridization and washing was complete, the bound, fluorescein-labeled probe was detected using an anti-fluorescein antibody as per the manufacturer's instructions with the kit. Similarly digested DNA from *Streptococcus pneumoniae* strain SJ2 (serotype 6B) was used as a positive control. Fluorescein-labeled bacteriophage lambda DNA digested 25 with the restriction nuclease *Hind*III was used as a size marker.

The Sp36 probe hybridized with a single fragment in the digested *S. aureus* DNA (~4.5 kb *Bam*HI fragment, ~5 kb *Pvu*II fragment) and with 2 major fragments in a *Pvu*II digest of serotype M1 of the group A 30 streptococci genomic DNA (~4.0 kb, and ~4.2 kb ).

**Example 2****BLAST Analysis Using Sp36 Predict d Amino Acid Sequ nce**

Sequence comparisons of the Sp36 encoded protein sequence against the publicly available GenBank sequence database (including the unfinished microbial database (<http://www.ncbi.nlm.nih.gov/BLAST/unfinishedgenome.html>)) revealed two highly homologous amino acid sequences. One of these was a predicted amino acid sequence from the *S. pyogenes* genome. This predicted polypeptide comprised 825 amino acid residues (MW = 92,616 Da) that was 25.1% identical to the Sp36 amino acid sequence from pneumococcus serotype 4 but maintained the 5 histidine triads (underlined in Figure 5(a) - SEQ ID NO: 2). The second polypeptide encoded within the *S. pyogenes* database contained several errors that were corrected by our sequencing of this region of the genome. The DNA fragment obtained encoded a protein of 792 amino acids (MW = 87,457 Da) that was 12.6% identical to the pneumococcal sequence and 12.5% identical to the first *S. pyogenes* polypeptide. This predicted amino acid sequence contained four histidine triad motifs (underlined in Fig. 5(b) – SEQ ID NO.: 4). The third polypeptide sequence obtained was one already in the database (Accession No. AF062533) and identified only as an unknown gene downstream from a gene identified as *Imb* in *S. galactiae*. This 822 amino acid protein thus has a predicted molecular weight of 92,353 Da and maintains the 5 histidine triad motifs (underlined in Figure 5(C) - SEQ ID NO: 6). This second polypeptide shows 25.6% sequence identity to Sp36 of pneumococcus type 4 and 97.7% and 11.6% identity to the two group A homologs, respectively.

**Example 3****Southern Blot Analysis Using a group A Streptococcal Sp36 Homolog Probe**

5        Southern blot analysis was performed with a fluorescein-labeled DNA  
fragment as probe, which encoding a group A streptococcal Sp36 homolog  
cloned from an M1 serotype of the group A streptococcal genome. This  
fragment was then used to probe genomic DNA from an M6 serotype of the  
group A streptococcal genome, as well as serotype 1a and serotype 3 of  
10      the group B streptococcal genome, and strain SJ2 (serotype 6B) of  
pneumococcus. In all cases, a single band was obtained in DNA digested  
with *Bam*HI when hybridization was carried out under low stringency  
conditions (as described above). A band of about 20 kb was visualized in  
group A streptococcal DNA, about 4.5 kb was obtained for group B  
15      streptococcal DNA, and a band of about 4kb was seen for pneumococcus.

**Example 4**

20

**Western Blot Analysis of Reactivity of group B Streptococcal Homolog With  
Anti-Pneumococcal Sp36 Antiserum**

25      To determine whether antiserum raised against recombinant Sp36  
from *S. pneumoniae* would recognize the recombinant Sp36 homolog  
encoded by group B streptococcal organisms, a western blot was  
performed. One hundred nanograms (100 ng) of recombinant Sp36  
polypeptide cloned from either *S. pneumoniae* serotype 4, or of the Sp36  
homolog cloned from group B streptococcal organisms, or from an unrelated  
30      recombinant protein control expressed and purified in the same way, were  
subjected to SDS-PAGE containing 12% acrylamide. A cell lysate of

pneumococcal strain SJ2 (serotype 6B) was also included on the gel. After electrophoresis, the separated proteins were transferred to a nitrocellulose membrane and probed with rabbit polyclonal antiserum raised against the recombinant pneumococcal protein. Bound antibodies were detected 5 chemiluminescently with a goat anti-rabbit IgG antibody conjugated to horseradish peroxidase using the substrate ECL (from Amersham). The results demonstrate that antiserum raised against the pneumococcal Sp36 protein cross-react with the Sp36 homolog identified from the group B streptococci and thereby indicating conservation of epitopes between the 10 proteins. The group B streptococcal homolog is also approximately the same size as the protein detected in *S. pneumoniae* lysates. Because the group A and B homologs are highly homologous, if not identical, such antiserum would also likely cross-react with the group A streptococcal protein.

15

#### Example 5

##### Alignment of Predicted Amino Acid Sequences of the Sp36 Homologs from group A and B Streptococci With Pneumococcal Sp36

20

The predicted amino acid sequences from the Sp36 genes from group A and group B streptococci and *S. pneumoniae* were aligned using the Clustal algorithm in a DNASTar Computer package (DNASTar, Inc., Madison, WI). Amino acids that match those encoded by the pneumococcal 25 gene are boxed in Figure 2 (showing the results of the alignment). Gaps introduced in the sequence by the alignment process are indicated by dashed lines.

30

#### Example 6

**Percentage Sequence Identity Between Homologs of Sp36**

The Sp36 amino acid sequence from pneumococci is 25.6% identical to the predicted amino acid sequence of the homologous gene of group B streptococci and 25.1% and 12.6% identical to the deduced sequences of the two genes from group A streptococci. Furthermore, the group B homolog is 97.7% and 11.6% identical to the first (GAS36) and second (GAS36(2)) homologs from group A streptococci, respectively. These experiments indicate that homologous genes to Sp36 from pneumococcus are present in group A and group B streptococci, as well as in *Staphylococcus aureus*. The protein encoded by this gene may therefore perform a similar function in these different organisms. This suggests that a vaccine comprising one or more of these proteins may be broadly protective against these species. These results are summarized in Table 1 which shows the percent identity between the amino acid sequences of Sp36 from pneumococcus strain Norway 4 (serotype 4), group A streptococci Sp36 homolog from an M1 serotype, and group B streptococci Sp36 from strain R268.

20 **Table 1.**

	Pneumo. Sp36	GAS36	GAS36(2)	GBS36
Pneumo. Sp36	100%	25.1%	12.6%	25.6%
GAS36	---	100%		97.7%
25 GAS36(2)	---	---	100%	11.6%
GBS36	---	---		100%

where GAS36 = SEQ ID NO: 2

30 GAS36(2) = SEQ ID NO: 4

GBS36 = SEQ ID NO: 6

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising an amino acid sequence at least 75% identical to a sequence selected from the group consisting of  
5 SEQ ID NO: 2, 4 and 6.
2. The isolated polypeptide of claim 1 wherein said polypeptide is at least 90% identical to the sequence selected from the group consisting of SEQ ID NO: 2, 4, and 6.  
10
3. The isolated polypeptide of claim 1 wherein said polypeptide is at least 95% identical to the sequence selected from the group consisting of SEQ ID NO: 2, 4, and 6.
- 15 4. The isolated polypeptide of claim 1 wherein said polypeptide has the amino acid sequence selected from the group consisting of SEQ ID NO: 2, 4 and 6.
- 20 5. The isolated polypeptide of claim 1 wherein said polypeptide is found in an organism selected from the group consisting of group A streptococci, group B streptococci, and *Staphylococcus aureus*.
- 25 6. The isolated polypeptide of claim 5 wherein the group A streptococcal organism is *Streptococcus pyogenes*.
7. The isolated polypeptide of claim 5 wherein the group B streptococcal organism is *Streptococcus agalactiae*.
- 30 8. The isolated polypeptide of claim 1 wherein said polypeptide has a sequence at least 25% identical to the amino acid sequence of the Sp36 protein of *Streptococcus pneumoniae*.

9. An isolated polynucleotide comprising a sequence coding for a polypeptide selected from the group consisting of the polypeptides of claims 1, 2, 3, 4, 5, 6, 7, and 8.

5

10. The isolated polynucleotide of claim 9 wherein said polynucleotide has a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 3 and 5.

10

11. An antibody specific for a polypeptide selected from the group consisting of the polypeptides of claims 1, 2, 3, 4, 5, 6, 7, and 8.

12. The antibody of claim 11 wherein said antibody is a monoclonal antibody.

15

13. A genetically engineered cell producing the antibody of claim 12.

14. A vector comprising the polynucleotide of claim 9.

20

15. A vector comprising the polynucleotide of claim 10.

16. A genetically engineered cell expressing the polypeptide coded for by the polynucleotide of claim 9 or 10.

25

17. A composition comprising a polypeptide selected from the group consisting of the polypeptides of claims 1, 2, 3, 4, 5, 6, 7, and 8, said polypeptide being suspended in a pharmacologically acceptable diluent or excipient.

30

18. A vaccine composition comprising a polypeptide selected from the group consisting of the polypeptide of claims 1, 2, 3, 4, 5, 6, 7, and 8,

said, polypeptide being present in an amount effective to produce an immune response, and wherein said polypeptide is suspended in a pharmacologically acceptable carrier, diluent or excipient.

5        19. A vaccine comprising an immunogenically active amount of the composition of claim 17.

10      20. A method of vaccinating an animal against infection by a bacterial organism selected from the group consisting of streptococcal bacteria and staphylococcal bacteria comprising administering to said animal an immunologically effective amount of the vaccine of claim 19.

21. The method of claim 20 wherein said animal is a human.

15      22. A method of treating a disease comprising administering to an animal afflicted therewith of a therapeutically effective amount of an antibody of claim 12 wherein said antibody is suspended in a pharmacologically acceptable carrier, diluent or excipient.

20      23. The method of claim 22 wherein said animal is a human.

24. The method of claim 22 wherein said disease is caused by an organism selected from the group consisting of group A streptococci, group B streptococci, and *Staphylococcus aureus*.

25

30

**Figure 1**

**Figure 2 (a)**

			10	20	30																												
1	V	K	K	T	Y	G	Y	-	I	G	S	V	A	A	I	L	L	A	T	H	I	G	S	Y	O	L	G	K	H	H		Gas36.pro	
1	V	K	K	T	Y	G	Y	-	I	G	S	V	A	A	I	L	L	A	T	H	I	G	S	Y	O	L	G	K	H	H		Gbs36.PRO	
1	M	K	I	N	K	K	L	V	G	S	A	A	A	L	L	S	-	-	V	C	S	Y	E	L	G	L	Y	O		Pneumo Sp36.PRO			
												40	50	60																			
30	M	G	S	A	T	K	D	N	O	I	A	Y	I	D	D	S	K	G	A	K	A	P	K	T	N	K	T	H	D		Gas36.pro		
30	M	G	L	A	T	K	D	N	O	I	A	Y	I	D	D	S	K	G	V	K	A	P	K	T	N	K	T	H	D		Gbs36.PRO		
29	A	R	T	V	K	E	N	N	R	V	S	Y	I	D	G	K	O	A	T	K	T	-	-	E	N	L	T	P	D		Pneumo Sp36.PRO		
												70	80	90																			
60	Q	I	S	A	E	E	G	I	S	A	E	O	I	V	V	K	I	T	D	O	G	Y	V	T	S	H	G	D	H	Y		Gas36.pro	
60	Q	I	S	A	E	E	G	I	S	A	E	O	I	V	V	K	I	T	D	O	G	Y	V	T	S	H	G	D	H	Y		Gbs36.PRO	
57	F	V	S	K	R	E	G	I	N	A	E	O	I	V	I	K	I	T	D	O	G	Y	V	T	S	H	G	D	H	Y		Pneumo Sp36.PRO	
												100	110	120																			
90	H	F	Y	N	G	K	V	P	Y	D	A	I	I	S	E	E	L	L	H	T	D	P	N	Y	R	F	K	O	S	D		Gas36.pro	
90	H	F	Y	N	G	K	V	P	Y	D	A	I	I	S	E	E	L	L	H	T	D	P	N	Y	H	F	K	O	S	D		Gbs36.PRO	
87	H	Y	Y	N	G	K	V	P	Y	D	A	I	I	S	E	E	L	L	H	K	D	P	N	Y	K	L	K	D	E	D		Pneumo Sp36.PRO	
												130	140	150																			
120	V	I	N	E	I	L	D	G	Y	V	I	K	V	N	G	N	Y	Y	V	L	K	P	G	S	K	R	K	N	I		Gas36.pro		
120	V	I	N	E	I	L	D	G	Y	V	I	K	V	N	G	N	Y	Y	V	L	K	P	G	S	K	R	K	N	I		Gbs36.PRO		
117	I	V	N	E	V	K	G	G	Y	V	I	K	V	D	G	K	Y	Y	V	L	K	D	A	A	H	A	D	N	Y		Pneumo Sp36.PRO		
												160	170	180																			
150	R	T	K	D	O	O	I	A	E	O	V	A	K	G	T	K	E	A	K	E	K	G	L	A	O	V	A	H	L	S	K		Gas36.pro
150	R	T	K	D	O	O	I	A	E	O	V	A	K	G	T	K	E	A	K	E	K	G	L	A	O	V	A	H	L	S	K		Gbs36.PRO
147	R	T	K	E	E	I	N	R	Q	-	K	O	E	H	S	O	H	R	E	G	G	T	P	R	-	-	-	-	-	-	-		Pneumo Sp36.PRO
												190	200	210																			
180	E	E	V	A	A	V	N	E	A	K	R	O	G	R	Y	T	T	D	D	G	Y	I	F	S	P	T	D	I	I	D		Gas36.pro	
180	E	E	V	A	A	V	N	E	A	K	R	O	G	R	Y	T	T	D	D	G	Y	I	F	S	P	T	D	I	I	D		Gbs36.PRO	
170	-	N	D	G	A	V	A	L	A	R	S	O	G	R	Y	T	T	D	D	G	Y	I	F	N	A	S	D	I	E		Pneumo Sp36.PRO		
												220	230	240																			
210	D	L	G	D	A	Y	L	V	P	H	G	N	H	Y	H	Y	I	P	K	K	D	L	S	P	S	E	L	A	A	A		Gas36.pro	
210	D	L	G	D	A	Y	L	V	P	H	G	N	H	Y	H	Y	I	P	K	K	D	L	S	P	S	E	L	A	A	A		Gbs36.PRO	
199	D	T	G	D	A	Y	I	V	P	H	G	D	H	Y	H	Y	I	P	K	N	E	L	S	A	S	E	L	A	A	A		Pneumo Sp36.PRO	
												250	260	270																			
240	Q	A	Y	W	S	O	K	O	G	R	G	A	R	P	S	D	Y	R	P	T	P	A	P	A	P	G	R	R	K	A		Gas36.pro	
240	Q	A	Y	W	S	O	K	O	G	R	G	A	R	P	S	D	Y	R	P	T	P	A	-	-	P	G	R	R	K	A		Gbs36.PRO	
229	E	A	F	L	S	G	R	-	G	N	L	S	N	S	R	T	Y	R	-	Q	N	S	D	N	T	S	R	T	N		Pneumo Sp36.PRO		
												280	290	300																			
270	P	I	P	D	V	T	P	N	P	G	G	H	O	P	D	N	G	G	H	P	A	P	P	R	P	N	D	A		Gas36.pro			
268	P	I	P	D	V	T	P	N	P	G	G	H	O	P	D	N	G	G	H	P	A	P	P	R	P	N	D	A		Gbs36.PRO			
257	W	Y	P	S	V	S	-	-	-	-	-	-	-	-	-	-	-	-	N	P	G	T	T	N	T	N	S		Pneumo Sp36.PRO				
												310	320	330																			
300	S	O	N	K	H	O	R	D	E	F	K	G	K	T	F	K	E	L	L	D	O	L	H	R	L	D	L	K	Y	R		Gas36.pro	
298	S	O	N	K	H	O	R	D	E	F	K	G	K	T	F	K	E	L	L	D	O	L	H	R	L	D	L	K	Y	R		Gbs36.PRO	
273	N	N	S	N	T	N	S	O	A	S	O	N	D	I	S	L	K	O	L	Y	K	L	P	L	S	O	R		Pneumo Sp36.PRO				

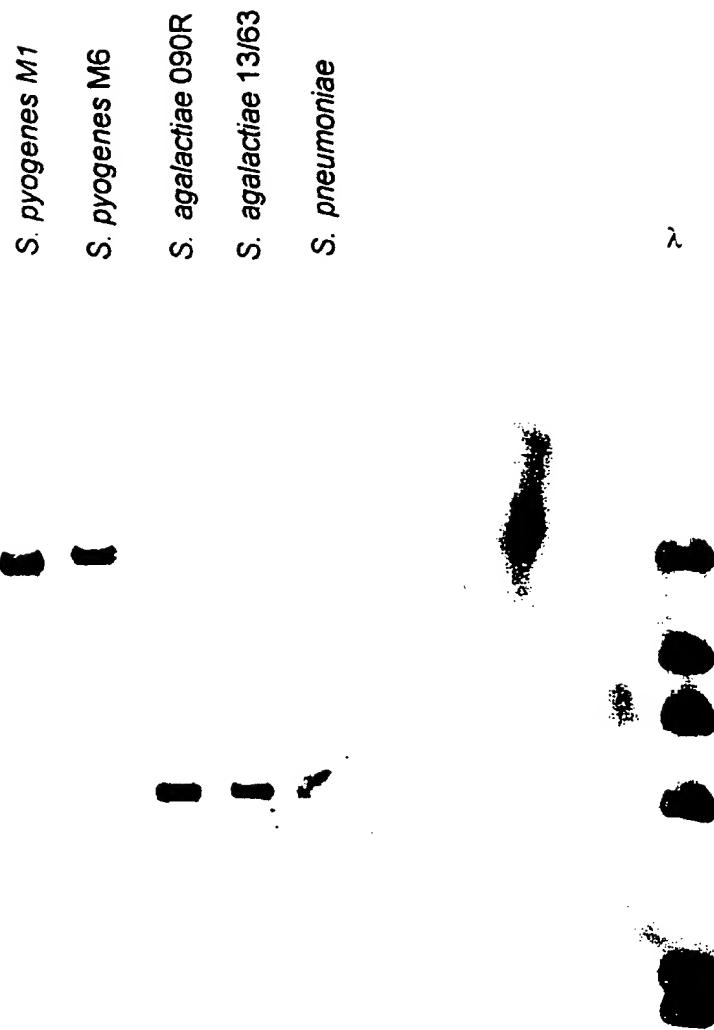
**Figure 2 (b)**

330	H V E E D G L I F E P T O V I K S N A F G Y V V P H G D H Y	340	350	360	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
328	H V E E D G L I F E P T O V I K S N A F G Y V V P H G D H Y				
303	H Y E S D G L V F D P A Q I T S R T A R G V A V P H G D H Y				
360	H I I P R S O L S P L E M E L A D - - - R Y L A G O - - -	370	380	390	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
358	H I I P R S O L S P L E M E L A D - - - R Y L A G O - - -				
333	H F I P Y S O M S E L E F E R I A R I I P L R Y R S N H W V P				
383	T E D D D S G S D H S K P S D K E V T H T F L G H R I K A Y	400	410	420	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
381	T D D N D S G S D H S K P S D K E V T H T F L G H R I K A Y				
363	D S R P E O P S P O P T P E P P S P G P O P A P N L K I D S N				
413	G K G L D G K P Y D T S D A Y V F S K E S I H S - V D K S G	430	440	450	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
411	G K G L D G K P Y D T S D A Y V F S K E S I H S - V D K S G				
393	S S L V S O L V R K V G E G Y V F E E K G I S R Y V F A K D				
442	V T A K H G D H F H Y - I G F G E L E O Y E L D E V A N W V	460	470	480	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
440	V T A K H G D H F H Y - I G F G E L E O Y E L D E V A N W V				
423	L P S E T V K N L E S K L S K O E S V S H T L T A K K E N V				
471	K A K G O A - - D E L A A A L D O E O G K E K P L F D T K K	490	500	510	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
469	K A K G O A - - D E L V A A L D O E O G K E K P L F D T K K				
453	A P R D O E F Y D K A Y N L L T E A H - - - K A L F E N K G				
499	V S R K V T K D G K V G Y M M P K D G K D Y F Y A R D Q I D	520	530	540	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
497	V S R K V T K D G K V G Y I M P K D G K D Y F Y A R Y Q I D				
480	R N S D F O A L D K L L E R L N D E S T N - - - K E K L V				
529	L T O I A F - - - A E O E L M L K D K K H Y R Y D I V D T	550	560	570	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
527	L T O I A F - - - A E O E L M L K D K K H Y R Y D I V D T				
506	D D L L A F L A P I T H P E R L G K P N S O I E Y T E D E V				
555	G I E P R I L A - - - V D V S S L P M H A G N A T Y D T G S S	580	590	600	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
553	G I E P R I L A - - - V D V S S L P M H A G N A T Y D T G S S				
536	R I - A O L A D K Y T T S D G Y I F D E H D I I S D E G D A				
582	F V I P H I D H I H V V P Y S W L T - R D O I A T I K Y V M	610	620	630	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
580	F V I P H I D H I H V V P Y S W L T - R D O I A T I K Y V M				
565	Y Y T P H M G H S H W I G K D S L S D K E K V A A Q A Y T K				
611	Q H P E V R P - - - D I W S K P G H E E S G S V I P N V T	640	650	660	Gas36.pro Gbs36.PRO Pneumo Sp36.PRO
609	Q H P E V R P - - - D V W S K P G H E E S G S V I P N V T				
595	E K G I L P P S P D A D Y K A N P T G D S A A I Y N R Y K				

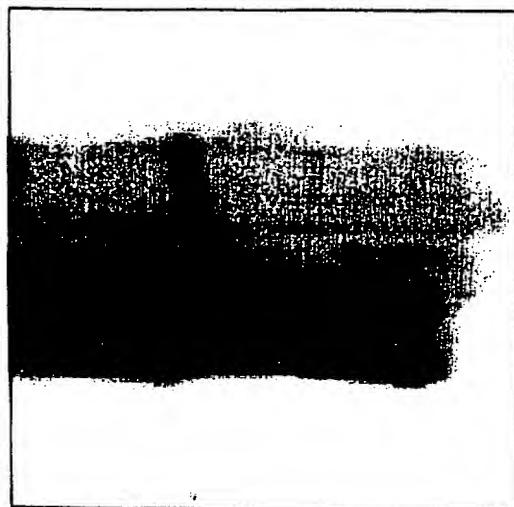
**Figure 2 (c)**

637	P L O K R A G H P N W O - I I H S A E E V O K A L A E G R F	Gas36.pro
635	P L D K R A G H P N W O - I I H S A E E V O K A L A E G R F	Gbs36.PRO
625	[G] - E K R I P L Y V R I P Y M V E H T V E Y K N G - - - - -	Pneumo Sp36.PRO
670                    680                    690		
666	A T P D G Y I F D P R D V L A K E T F V W - K D G S F S I P	Gas36.pro
664	A A P D G Y I F D P R D V L A K E T F V W - K D G S F S I P	Gbs36.PRO
648	- - - [N] L I P H K D H Y H N I K F A W F D D H T Y K A P	Pneumo Sp36.PRO
700                    710                    720		
695	R A D G S S L R T I - - - - - - - - - N K S D L S S O A E	Gas36.pro
693	R A D G S S L R T I - - - - - - - - - N K S D L S S O A E	Gbs36.PRO
674	- - [N] G Y T I L E D L F A T I K Y Y V E H P D E R P H S N O G	Pneumo Sp36.PRO
730                    740                    750		
714	[W] O O A O D E - L L A K K N A G D A T D T D - K P K E K Q O A	Gas36.pro
712	[W] O O A O D E - L L A K K N A G D A T D T D - K P E E K Q O A	Gbs36.PRO
702	W G N A S E H V L G K K D H S E D P N K N F K A D E E P V E	Pneumo Sp36.PRO
760                    770                    780		
742	D K S N E N O O P - - - S E A S K E E E K E S D D F I D S L	Gas36.pro
740	D K S N E N O O P - - - S E A S K E E - K E S D D F I D S L	Gbs36.PRO
732	E T P A E P E V P O V E T E K V E A O L K E A E V L L A K V	Pneumo Sp36.PRO
790                    800                    810		
769	P D Y G L D R A T T L E D H I N G Q L A O K A N I D P K Y L I F	Gas36.pro
766	P D Y G L D R A T T L E D H I N G Q L A O K A N I D P K Y L I F	Gbs36.PRO
762	T D S S L - K A N A T E T L A G I R N N L T L O - - - - [I] H	Pneumo Sp36.PRO
820                    830                    840		
799	O P E G V Q F Y N K N G E L V T Y - - - D I K T L O Q I N	Gas36.pro
796	O P E G V O F Y N K N G E L V T Y - - - D I K T L O Q I N	Gbs36.PRO
787	D N N S I M - - A E A E K L L A L L K G S N P S S Y S K E K	Pneumo Sp36.PRO
850                    860                    870		
825	P	Gas36.pro
822	P	Gbs36.PRO
815	I N	Pneumo Sp36.PRO

**Figure 3**



## Figure 4



Control

Sp36 GBS

Sp36

SJ2 6b

Figure 5 (a)

MKKTYGYIGS VAAILLATHI GSYQLGKHHM GSATKDNOIA YIDDSKGKA K APKTKNTMDQ ISAEEGISAE 70  
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 RPGSKRKNIR TKQQIAEQVA KGTKEAKEKG LAQVAHLSKE EVAAVNEFAKR OGRTYTTDDGY IFSPTDIIDD 210  
 LGDAYLVPHG NHIIHYIPKKD LSPSELAAAO AYWSQKQGRG ARPSDYRPTP APAPGRRKAP IPDVTPNPQG 280  
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 YDTSDAYVFS KESIHSVDKS GTATAKHGDHF HYICFGELEQ YELDEVANTW KAKGQADELLA AALDQEQQKE 490  
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 VIPNVUTPLDK RAGMPNWQII HSAEEVQKAL AEGRFATPDG YIFDPRDVLA KETFWKDGS FSIPRADGSS 700  
 LRTINKSDLS QAEMWQQAQEL LAKXNAGDAT DTDKPKEKQQ ADKSNNENQQP SEASKEEEEKE SDDFIDS LPD 770  
 YGLDRATLED HINQLAQKAN IDPKYLIFQP EGVQFYNNKNG ELVTDYDIKTL QQINP 825

Figure 5(b)

MTKKVIILV GLLSSQLTL IACQSRGNGT YPIKTKQSRK GMTSNKIKPI KKSKKTNKTH KGVAGVDFPT 70  
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### Figure 5 (c)

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 TINKSDLSQLA EWQQAQELLA KKNAGDATDT DKPEEKQQAD KSNENQQPSE ASKEEKESDD FIDSLPDYGL 770  
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## SEQUENCE LISTING

<110> Heinrichs, Jon  
Johnson, Leslie S.  
Koenig, Scott  
Adamou, John E.

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Pro Tyr Asp Ala Ile Ile Ser Glu Glu Leu Leu Met Thr Asp Pro Asn  
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## INTERNATIONAL SEARCH REPORT

Intern. Appl. No.

PCT/US 00/23417

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C07K14/315 C12N15/31 C07K16/12 A61K39/09 A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 18930 A (HUMAN GENOME SCIENCES INC ;CHOI GIL H (US); HRMOKYJ ALEX (US); J) 7 May 1998 (1998-05-07) SEQ ID NO:55 page 59 ---	8,9, 11-24
X	SPELLERBERG B ET AL: "Streptococcus agalactiae Lmb (lmb) gene, complete cds; and unknown gene" EMBL NUCLEOTIDE SEQU, 11 February 1999 (1999-02-11), XP002125180 cited in the application the whole document ---	1-5,7-10 -/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## • Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

9 January 2001

Date of mailing of the international search report

18.01.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

Billang, J

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/23417

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 42588 A (BIOCHEM VACCINS INC ; BRODEUR BERNARD R (CA); CHARLEBOIS ISABELLE () 26 August 1999 (1999-08-26) figures 3A, 3C -----	1-3,5,8, 9,11-24
P,X	WO 00 06736 A (HANNIFFY SEAN BOSCO ; LE PAGE RICHARD WILLIAM FALLA (GB); WELLS JER) 10 February 2000 (2000-02-10) clone 18 figure 1 -----	1-3,5, 7-9, 11-24

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/23417

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 20–24 are directed to a method of treatment of the human/animal body and/or a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of Invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-24, all partially

Polypeptide having SEQ ID NO: 2 and gene encoding the polynucleotide (SEQ ID NO: 1); antibodies specific for said polypeptide; vector and cells comprising said polynucleotide; composition comprising said polypeptide and methods making use of said polypeptide or antibodies.

2. Claims: 1-24, all partially

Polypeptide having SEQ ID NO: 4 and gene encoding the polynucleotide (SEQ ID NO: 3); antibodies specific for said polypeptide; vector and cells comprising said polynucleotide; composition comprising said polypeptide and methods making use of said polypeptide or antibodies.

3. Claims: 1-24, all partially

Polypeptide having SEQ ID NO: 6 and gene encoding the polynucleotide (SEQ ID NO: 5); antibodies specific for said polypeptide; vector and cells comprising said polynucleotide; composition comprising said polypeptide and methods making use of said polypeptide or antibodies.

## INTERNATIONAL SEARCH REPORT

## Information on patent family members

International Application No

PCT/US 00/23417

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9818930	A 07-05-1998	AU 5194598	A	22-05-1998
		AU 6909098	A	22-05-1998
		EP 0942983	A	22-09-1999
		EP 0941335	A	15-09-1999
		WO 9818931	A	07-05-1998
		US 6159469	A	12-12-2000
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WO 9942588	A 26-08-1999	AU 2505999	A	06-09-1999
		EP 1054971	A	29-11-2000
		NO 20004161	A	19-10-2000
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WO 0006736	A 10-02-2000	NONE		-----
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